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14. ABSTRACT This grant supported the initial development of a novel biochemical system, in vitro transcriptional networks, that can be programmed to construct arbitrary circuits. These systems are a simplification of genetic regulatory networks. The foundational theory is completed; design, implementation, and characterization, and optimization of individual switches has been performed; and construction of non-trivial biochemical test circuits is still in progress.					
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FINAL TECHNICAL REPORT

GRANT #: N00014-01-1-0813

PRINCIPAL INVESTIGATOR: Dr. Erik Winfree

INSTITUTION: California Institute of Technology

GRANT TITLE: "Biomolecular Computing by *in vitro* Transcriptional Networks"

AWARD PERIOD: 1 May 2001 – 30 April 2004

OBJECTIVES: Consider the challenge of creating biochemical computers, potentially no more than a few cubic microns in size (no bigger than a bacterium) and using only a few kT per logical operation. Biochemical computers of this sort will ultimately be pervasive for control of molecular and chemical processes – much as electrical computers today are pervasive for control of electro-mechanical devices – such as sensing, detecting, and classifying complex chemical milieu, controlling molecular nanofabrication processes, and serving as "brains" for future nanorobots.

Our approach toward this goal is to design *in vitro* biochemical systems, involving a small number of nucleic acid and enzyme species, capable of executing logical operations analogous to genetic regulatory networks in biological cells. We are proceeding in three steps: first, to develop DNA switches that can control transcription by RNA polymerase; second, to show that a dynamical steady-state is achieved in the presence of Ribonuclease; and third, to demonstrate that several switches can co-exist and interact in a single biochemical reaction, thus forming a circuit.

APPROACH: Models of simple genetic regulatory networks, in which DNA binding proteins either activate or repress expression of their target genes (which may code for other binding proteins), are formally equivalent to Hopfield's continuous rate equations for neural networks. Thus, these biochemical reactions are in principle capable of arbitrarily complex, robust information processing and pattern recognition tasks.

To explore how to program biochemical reactions, we create an *in vitro* analog of genetic regulatory networks using only two enzymes in addition to DNA and RNA molecules: T7 RNA polymerase (RNAP) and ribonucleases (RNase One, RNase R, and RNase H). RNAP transcribes synthetic DNA templates to produce RNA transcripts, which in turn function as "binding proteins" to regulate transcription of other DNA templates. The concentrations of RNA transcripts are the logical signals in the circuits. Regulations of transcription is controlled by binding of the RNA to the DNA template, changing its secondary structure to expose or hide the promoter sequence; only templates with exposed promoter sequences will be transcribed and produce RNA. Signals must turn off as well as turn on; this is the function of the ribonuclease.

Computation networks consist of several mutually interacting species in the same reaction. We have chosen, as a target for demonstrating the effectiveness of our approach, to implement a two gate network, (a binary flip-flop memory), and a three-gate recurrent network, (a 3 node ring oscillator).

ACCOMPLISHMENTS: Initial work developed and characterized single transcriptional switches, and examined their performance when one is used to drive the other. We have identified a switch design that is simpler than and outperforms our previous designs; optimized the design to improve the uniformity of its output transcript, its transcription rate, its switching time, and the efficacy of multiple on/off cycles; and examined the performance of several switches when both RNA polymerase and RNase are present; and determined that the reaction can be run in steady-state for a least ten hours. Furthermore, we have demonstrated that the output of one switch can be used as the input of the subsequent switch when both switches are present simultaneously in the same *in vitro* reaction.

Note that a key property of our design is that switch specificity is encoded by Watson-Crick complementarity of the RNA inhibitor and DNA template, and thus it should be straightforward to implement different logical networks incorporation many DNA and RNA species. The input domain of a switch is upstream of the promoter region and the output domain is downstream of the promoter region. This separation of domains allows design of modules to be combined later. It is important to note that arbitrary sequences, unless they have undesirable intra/intermolecular interactions, are qualified to be signals of *in vitro* circuit.

Initial attempts to construct the memory circuit and the oscillator circuit indicated that they operate correctly at the start, but after a few hours the switches stop functioning. We attribute this to the build-up of side-products and partial degradation products that interfere with the desired molecular interactions. In the final year of ONR funding, we have therefore focused on (a) evaluation and optimization of RNA degradation by ribonucleases One, H, and R, and (b) development of a fluorescence read-out system for precise real-time measurement of switch activity and transcript concentration. These investigations have lead to significant improvements in the operation of the switches, such that we expect that both target circuits soon will be functioning properly.

Additionally, we have completed our theoretical analysis of this circuit, showing how neural networks, digital circuits, and winner-take-all elements can be implemented using RNA transcriptional networks. This has been essential for understanding which characteristics (such as binding constants and Michaelis-Menton constants) are important for the functional behavior of transcriptional circuits.

CONCLUSIONS: In this project we have made fundamental progress in developing the components that will allow the construction of arbitrary biochemical transcriptional circuits *in vitro*.

PATENT INFORMATION: None

AWARD INFORMATION: None

REFEREED PUBLICATIONS (for total award period):

1. Jongmin Kim, John J. Hopfield, and Erik Winfree, "Neural network computation by *in vitro* transcriptional circuits," NIPS (to appear, 2004).

BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS (for total award period)

1. Jongmin Kim, Kristen Shantz, and Erik Winfree. "In Vitro Transcriptional Circuits," (in preparation).
2. Jongmin Kim, David Yu Zhang, and Erik Winfree. Abstract for DNA Based Computer 9 (June 1-4, 2003): "In Vitro Transcriptional Circuits."